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Impact of Exposure of Biomass Burning Emissions on Ovarian Activity and Reproductive Efficiency Indices in Cyclic and Non-Cyclic Crossbred Cows

[1]Heba, F. Hozyen, [2]G.E.S. Essawy, [2]M.A. Abdelrahman,
[1]Amal R. Abd El Hameed and [1]S.I.A. Shalaby

[1]Department of Animal Reproduction and AI, National Research Centre, Giza, Egypt
[2]Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract: This study aimed to detect the effect of rice straw burning emissions on some metabolic and hormonal parameters as well as its effect on some reproductive indices in local crossbred cows under field condition in Egypt. About 203 local cross-breed cows- reared at villages of Al-Sharkia governorate, Egypt - were divided into two main groups, control group included 90 cows reared in areas free from rice straw burning and second group were exposed to rice straw combustion products during the period of rice straw burning (from September to November). Decreased serum cholesterol value and progesterone level were obvious in air pollutants exposed cows. While, estradiol-17β level, total lipids and triglycerides values were not significantly affected. Days open, number of services per conception and incidence of smooth inactive ovaries were higher in exposed than non-exposed cows. In conclusion, ovarian activity and reproductive efficiency in cows exposed to rice straw burning emissions were adversely affected.

Key words: Air Pollution · Rice Straw · Burning · Ovarian Activity · Reproductive Efficiency Cattle

INTRODUCTION

Emissions from biomass burning are likely associated with adverse health effects [1]. In Egypt, it was reported that the average concentration of gaseous pollutants emitted in villages during the open burning of rice straw and close to the burning zone are much higher than those recorded in both urban and industrial areas in Cairo city [2]. Emissions released from rice straw combustion are a complex mixture of particulate matter (PM) and gaseous pollutants [3]. In addition, it was reported that rice straw burning is an important source of polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and volatile organic compounds which are produced by incomplete combustion of organic compounds and high-pressure processes [4].

The association of biomass burning exposure and lipid profile or dyslipidemia is still unknown. Setiawan *et al*. [5] reported that the level of cholesterol was lower and triglyceride was higher significantly in serum of rats inhaled to coal dust at 25mg/m3. Also, Reed *et al.* [6] recorded a significant decrease in serum cholesterol of mice inhaled biomass smoke based on PM concentration (30, 100, 300 and 1000 µg/m3) for 6 hours daily for 1 week. However, its value was significantly increased after 6 months exposure. While, Male albino rats subjected to 10 ppm SO_2 for 1 hour daily for 6 weeks showed an increase in blood total cholesterol and triglyceride values [7].

Reproductive system likely to be a target for endocrine disruption, particularly by estrogenic endocrine disrupters and harm the integrity of reproductive function in mammals and ultimately contribute to infertility [8]. Some environmental toxicants function as endocrine disruptors and therefore bind to hormone receptors without eliciting hormone-induced physiological events. Clotfelter *et al*. [9] and Miller *et al*. [10] reported that PAHs and its derivatives have been reported as environmental estrogens that could disrupt the hypothalamic-pituitary-gonadal axis and thus affect ovulatory cycle and fertility.

Corresponding Author: Amal R. Abd El Hameed, Department of Animal Reproduction and AI, National Research Centre, Giza, Egypt.

Some air pollutants have been linked to reproductive and developmental problems [11]. Baladi ewes exposed to rice straw combustion smoke twice a week for 1 year did not show any signs of estrous activity during both breeding and non breeding seasons with pale dry mucous membranes of external genital organs. Moreover, decreased plasma progesterone level with failure of conception in exposed as compared to non-exposed ewes [12]. In addition, Nykamp *et al*. [13] found that PAHs have the ability to decrease progesterone production in rat corpus luteal cells. Moreover, Archibong *et al*. [14] demonstrated that female mice dosed with benzo-a-pyrene sustained a decrease in ovarian corpora lutea numbers and volume.

The health hazards of biomass smoke remain uncertain (fuzzy). To date, there have been no reported studies to investigate the contributions of environmentally relevant levels of rice straw burning pollutants to health effects in cows. The current study aimed to monitor the effect of rice straw burning emissions on the key metabolic and hormonal parameters as well as the influence of such burning on some indices of reproductive efficiency in local crossbred cows under field condition in Egypt.

MATERIALS AND METHODS

Animals and Experimental Design: This study was conducted on a total number of 203 cows that were reared in small holder farms in Lower Egypt. Animals were chosen according to number of calving (1-3 calving) with an age ranged between 3-5 years and the age of animals was determined according to case history and dentition. Animals were apparently healthy and free from external parasites. According to exposure to rice straw burning products, cows were divided into two main groups; the control group included 90 cows that were reared in areas free from rice straw burning (approximately far more than 40 km from the burning zone) and the second group, cows that were directly exposed to rice straw combustion products. Cows in each group were subdivided according to ovarian activity (which inspected by rectal and ultrasonographic examinations) into; animals in follicular phase, animals in luteal phase and animals having bilateral smooth inactive ovaries.

Case History Included: number of previous calving, the date of last calving, the date of post-partum estrous, the number of services per conception and the last estrous cycle. These data were used for calculation of reproductive efficiency indices.

Sampling: Blood samples were collected from each animal by jugular venipuncture and put it in tubes without anticoagulant and left to clot for serum separation. After centrifugations at 3000 rpm for 10 minutes. Collected serum samples were kept at -20° C till be used for measurement of lipids and hormonal assays.

Lipid Profile: Serum total lipids, cholesterol and triglycerides concentrations were determined spectrophotometrically according to the methods of Zollner and Kirsch [15], Finley [16] and Fassati and Prencipe [17] respectively.

Serum Ovarian Steroids: Serum estradiol-17β and progesterone levels were assayed in duplicates by means of ELISA microwells procedures of Hall [18] and Tietz [19] respectively.

Reproductive Efficiency Parameters: Reproductive efficiency indices were calculated from the recorded data of case history taking in consideration the scheme of Youngquist [20] as follows:

Days Open (Calving Conception Interval): Days open was calculated as the number of days from calving to the current date for open cows.

Number of Services per Conception: Average number of services per conception was calculated by dividing the total number of breeding by the number of cows.

Incidence of Smooth Ovaries: Incidence of animals with bilateral smooth ovaries was calculated as a percentage from the total number of investigated cows.

Statistical Analysis: Data were analyzed statistically for lipids measurement and hormonal assays by 2-way analysis of variance using SPSS 16.0 for windows. Reproductive efficiency parameters were analyzed by student t-test according to Snedecor and Cochran [21]. Treatment means were compared by the least significance difference (LSD) at 5% level of probability.

RESULTS

Lipid Profile: It is evident from values shown in Table (1) that exposure to burning emissions induced a significant ($P<0.05$) decrease in serum cholesterol in exposed cows when compared to non-exposed (150.3 versus 184.8). Furthermore, serum cholesterol was lower in non-cyclic cows when compared to cyclic cows.

Table 1: Effect of exposure of cows to air pollution during different ovarian activities on serum total lipids (mg/dL), cholesterol (mg/dL) and triglycerides

Parameter	Total lipids(mg/dl)			Cholesterol(mg/dl)			Triglycerides(mg/dl)		
Groups	Non exposed	Exposed	Overall mean	Non exposed	Exposed	Overall mean	Non exposed	Exposed	Overall mean
Follicular phase	504.6±10.9	487.4±17.5	496.0	212.9±8.2	156.3±3.7	184.6A	6.2±0.3	6.3±0.1	6.3
Luteal phase	508.6±18.1	469.9±12.2	489.3	179.1±4.0	151.4±6.2	165.3A	6.1±0.2	6.6±0.2	6.4
Smooth inactive ovaries	466.0±27.0	455.9±18.3	460.9	162.5±13.0	143.2±4.3	152.9B	6.6±0.2	6.4±0.2	6.5
Overall mean	493.1	471.04		184.8a	150.3b		6.3	6.5	

-Data presented as means ± SE. N = 8 per group.

-Means having different superscripts within the same column or raw are significantly different at P< 0.05.

-LSD of exposure to air pollution (cholesterol) = 12.09.

-LSD of ovarian activity (cholesterol) = 14.81.

-LSD of interactions between air pollution and ovarian activity (cholesterol) =20.95

Table 2: Effect of exposure of cows to air pollution during different ovarian activities on estradiol-17β level (pg/ml) and progesterone (ng/ml) levels

Parameter	estradiol-17β (pg/ml) level			progesterone (ng/ml) level		
Groups	Non exposed	Exposed	Overall mean	Non exposed	Exposed	Overall mean
Follicular phase	12.6±0.95	12.1±0.92	12.35A	0.49±0.003	0.48±0.005	0.49B
Luteal phase	5.8±0.49	5.5±0.33	5.65B	6.56±0.56	4.50±0.29	5.46A
Smooth inactive ovaries	2.6±0.12	2.3±0.17	2.45B	ND	ND	0.0B
Overall mean	7.00	6.70		2.17a	1.71b	

-Data presented as means ± SE. N = 8 per group.

-Means having different superscripts within the same column or raw are significantly different at P> 0.05.

-ND= non detectable.

Table 3: Effect of exposure of cows to air pollution on reproductive efficiency parameters

	Non exposed	Exposed
Days open	125.0±10.31	196.7±11.94**
Services per conception	1.67±0.13	1.71±0.14
Incidence of smooth ovaries	20.3% (N=90)	38.7%** (N=113)

Data presented as means ± SE, N = 24 per group

** Significant at P<0.01.

However, no significant changes could be traced between air pollution and ovarian activity. In contrast, the overall means of serum total lipids and triglycerides were not significantly affected by exposure to burning emissions, different ovarian activities or interaction between burning emissions and ovarian activity.

Serum Ovarian Steroids: As shown in Table (2), the overall means of estradiol-17β in pollutants exposed cows are not affected significantly by rice straw combustion. While, significant (P<0.05) changes among different ovarian activities were observed. Follicular phase showed the highest value while luteal and smooth inactive ovaries showed lower value without significant difference between them. However, no significant changes could be traced due to interaction between air pollution and ovarian activity. On the other hand, exposure to burning emissions was associated with a significance (P<0.05) decrease in overall mean of progesterone level that was mostly noticeable during luteal phase. Also, there were significant (P<0.05) changes due to interaction between air pollution and ovarian activity while, studied stages were not significantly different.

Reproductive Efficiency Parameters: It is clear from Table (3) that pollutants emitted from rice straw burning have negative drawback on reproductive efficiency of exposed cows with reference to non exposed cows. Exposed cows exhibited a significantly (P<0.01) longer days open (196.7 days versus 125.0 days), slight increase in number of services per conception (1.71 versus 1.67) without significance difference. Higher incidence of bilateral smooth ovaries in animals exposed to burning emission (38.7% versus 20.3%).

DISCUSSION

Exposure to biomass burning emissions is common in the environment and has been reported as a health concern. Rice is one of the most important crops in Egypt, especially in the northern east part of the country.

For each ton of produced rice, two tons of rice straws are disposed. Burning of straw as well as other agricultural waste residues is a common practice for their disposal [22].

Reproductive system and its endocrine and neural controls are susceptible to alteration following environmental exposure to variety of agents [23].

Cholesterol is the structural component of all cell membranes, modulating their fluidity and in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones and vitamin D [24]. In our study, cholesterol concentrations in cows exposed to rice straw burning emissions were lower than non-exposed animals but no significant changes occurred in both triglycerides and total lipids. Similar results of low cholesterol were obtained in the studies of Reed *et al.* [6] in mice and Setiawan *et al* [5] in rats. On the other hand, Agar *et al.* [7] recorded increased blood total cholesterol and triglyceride values in rats. Meanwhile, Setiawan *et al*. [5] suggested that the mechanism by which (PM10)of coal dust inhalation lowers cholesterol may be related to increased reverse cholesterol transport (RCT) by which cholesterol is transported from the peripheral tissues and cells to the liver.

Cows exposed to air pollutants showed a significant decrease in serum progesterone level during the luteal phase when compared to non-exposed cows while; the estrogen levels were not significantly altered. Cholesterol is a precursor of steroid hormones [24] and high value of cholesterol in cyclic animals is an indicative of more secretion of steroids during estrous due to increased ovarian activity [25]. This decrease in progesterone implies a reduction in the metabolic activities responsible for ovarian steroid synthesis and release by luteal tissue [14]. Also, El-Tohamy *et al*. [26] attributed the inferior postpartum reproductive parameters to changes in some blood parameters whereas they noticed that cows suffered from ovarian hypoactivity have low blood concentration of cholesterol.

Moreover, PAHs and its derivatives have been reported as environmental estrogens that could disrupt the hypothalamic–pituitary–gonadal axis and thus affect estrogen level and fertility [10]. Also, decreased progesterone production may be as a result of reactive oxygen species (ROS) generated by PAHs. Redox cycling of PAHs in mitochondria, but also in the cytoplasm would be the source of ROS. As for steroidogenic steps vulnerable to ROS, one site could be the cytochrome P450 side chain cleavage site in mitochondria where cholesterol is metabolized to pregnenolone [13]. This step is subject to free radical control [27]. An alternative or additional mechanism to explain how PAHs could disrupt progesterone secretion is via its effect on LH signal transduction. LH stimulates secretion of progesterone by binding to its receptor on the plasma membrane of the corpus luteum cells and activates the cAMP intracellular messenger pathway. In turn, cyclic AMP causes phosphorylation of proteins that are involved in steroid hormone synthesis [28]. Interference with any of these steps could reduce progesterone secretion. H_2O_2, which would arise from the redox cycling of PAHs, has been shown to inhibit LH stimulated cAMP accumulation in luteal cells by possibly uncoupling LH receptors through interruption of G-protein dependent activation of adenylate cyclase [29]. In this respect, decreased progesterone levels may be related to cessation of ovarian function following loss of body condition and negative energy balance after disturbance of the general health status and consequently reduced LH concentration, decreased steroid secretion rate with low quality oocyte [12]. PAHs may cause toxicity in the ovary after metabolic activation to electrophilic intermediates capable of covalent binding to cellular macromolecules such as DNA, RNA, or proteins. The first step in metabolic activation occurs via a microsomal P450-dependent monooxygenase, aryl hydrocarbon hydroxylase. The epoxides formed may either spontaneously rearrange, undergo hydrolysis by epoxide hydrase or become conjugated by related enzymes. This study revealed a low cholesterol level as well as low progesterone values with lower reproductive performance. Burle *et al*. [30] and Umesh *et al*. [31] observed that there is a positive correlation between higher cholesterol concentration and better reproductive performance.

In the present study, exposure of cows to pollutants emitted from rice straw burning was associated with a negative drawback on reproductive efficiency. Whereas, these animals in comparison with non exposed cows had significantly longer days open (196.7 days versus 125 days), slight increase of the number of services per conception (1.71 versus 1.67) and high incidence of animals with smooth ovaries (38.7% versus 20.3%). Similar findings were recorded by Waldner and Stryhn [32] in cows exposed to high levels of sulfur dioxide, volatile organic compounds emissions from oil-and gas-producing facilities as they revealed an increase of open days and subsequently calving interval. Likewise, Waldner [33] reported an increase in the time from breeding to calving and calving interval associated with exposure of cows to volatile organic compounds measured as benzene during the first month of bull contact. Ewes exposed to rice straw

burning emissions suffered from failure of conception that was attributed to cessation of ovarian function following loss of body condition and negative energy balance after disturbance of the general health status and consequently reduced LH concentration, decreased steroid secretion rate with low quality oocyte [12].

Also, low peripheral concentrations of progesterone after insemination are related to low fertility of cows. However, low fertility in cattle is also related to low progesterone concentrations during the estrus cycle preceding insemination [34]. In addition, Ahmed *et al*. [35] reported low total lipids, triglycerides and cholesterol concentrations was associated with ovarian inactivity in cows as negative energy balance not only suppresses pulsatile LH secretion, but also reduces ovarian responsiveness to LH stimulation with decreased plasma glucose and insulin levels.

In conclusion, this study suggested that straw burning emission can produce important metabolic disturbances based on alterations of lipid profile and decrease progesterone. Exposed cows revealed inferior reproductive potentials.

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